Strong genome-wide divergence between sympatric European river and brook lampreys

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Lampreys, together with hagfishes, are the only extant representatives of jawless vertebrates and thus of prime interest for the study of vertebrate evolution [1]. Most lamprey genera occur in two forms with divergent life histories: a parasitic, anadromous and a non-parasitic, freshwater resident form [2–8]. The taxonomic status of such 'paired species' is disputed, however. While indistinguishable at larval stages, but clearly distinct as adults, they cannot be differentiated with available genetic data [6,7], which has fuelled speculations that the two forms may in fact represent products of phenotypic plasticity within a single species. Here, we use restriction site-associated DNA sequencing (RADseq) to examine the genetic population structure of sympatric European river (*Lampetra fluviatilis* L., 1758) and brook (*Lampetra planeri* Bloch, 1784) lampreys. We find strong genetic differentiation and identify numerous fixed and diagnostic single nucleotide polymorphisms (SNPs) between the two species, 12 of which can be unequivocally assigned to specific genes.

Lampreys — often referred to as cyclostomes because of their circular mouth — commonly occur as species pairs with distinct post-larval life histories. The so-called brook lampreys spend their entire life in freshwater, whereas their parasitic counterparts, the river lampreys, spend most of their adult life in the ocean or in estuaries and return to freshwater only for reproduction [2–8]. Whether these two forms are real species or are products of phenotypic plasticity in a single species has puzzled biologists for decades [2–4]. In the adult stage, river lampreys are much larger and morphologically distinct from brook lampreys, which is why they have been described as distinct species. On the other hand, the larvae of the two forms are indistinguishable, the adults co-occur on breeding grounds and often spawn in common nests [8], and they produce viable offspring when crossed artificially [4], lending support to the plasticity hypothesis. Importantly, no genetic evidence is available to date that would suggest their separation (e.g., [6,7]). Sympatric European *L. fluviatilis* and *L. planeri* even share mitochondrial haplotypes, which was suggested to reflect ongoing gene flow or, alternatively, incomplete sorting of ancestral polymorphisms [6].

To address this 'paired species' conundrum in lampreys, we examined one pair in detail by means of Illumina-sequenced RAD. We considered 17 specimens of *L. fluviatilis* (Figure 1A) and 18 specimens of *L. planeri* (Figure 1B) collected from a common spawning site in the Sorraia River, a tributary of the Tagus River in Portugal, the southern limit of their distribution (see Supplemental Information published with the online version of this article). Sequences from one individual were used to build a pseudo-reference genome spanning 39,865 RAD loci (3.79 Mb), against which all individuals were aligned. Screening the alignments recovered 8,826 polymorphic RAD loci, yielding a total of 14,691 informative SNPs.

Global F_{ST} based on all SNPs between the two sympatric lampreys was no less than 0.37, suggesting strong genome-wide genetic differentiation despite the shared mitochondrial DNA haplotypes reported earlier for the exact same system [6]. Likewise, a genetic assignment test using Structure unambiguously separated the surveyed individuals into two distinct clusters (Figure 1C). The same result was obtained when the SNPs were analyzed in a phylogenetic context (Figure 1D). We thus provide the first genetic evidence for the taxonomic validity of the two European lamprey species *L. fluviatilis* and *L. planeri*. At the same time, we highlight the power of next generation sequencing technologies to resolve old questions in biology. Our data further agree with the assumption that resident lampreys are derived from migratory ones [2,3]. The genome scan revealed much greater genetic diversity in *L. fluviatilis* than in *L. planeri.* For instance, *L. fluviatilis* displayed a 42% higher density of private SNPs than *L. planeri* (7,399 *versus* 5,198; binomial $p < 0.001$; see also branch-lengths in Figure 1D). In addition, the greater genetic diversity in the migratory species might also reflect the larger effective population size and less restricted gene flow. By contrast, we expect resident species to be more prone to genetic bottlenecks and genetic drift due to their reduced mobility.

To gain insight into genes potentially underlying the divergence between the sympatric lampreys, we screened the marker data for loci fixed for different alleles between the two species ($F_{ST} = 1$), identifying 166 such distinctive SNPs. Making use of the recently published genome of the sea lamprey [1], a distant relative of the species under investigation, we subjected these loci to reciprocal BLAST searches. This allowed us to link 12 of these loci to annotated genes. Interestingly, most of the genes showing fixed allelic differences between the two lampreys are related to functions that have previously been implicated in the adaptation to a migratory versus resident life-style in lampreys and bony fishes. For instance, fixed differences were found in the vasotocin gene, a major player in saltwater–freshwater osmoregulation and also involved in life history divergence [9], and in the gonadotropin-releasing hormone (GnRH), a key gene in gonadal development and differentiation [10]. We also found fixed genetic differences in four genes related to immune functions, three axial patterning genes, a pineal-gland-specific opsin, a sodium channel gene, and a tyrosine phosphatase gene. These genes are likely to contribute to ecologically based reproductive isolation in this lamprey system, paving the way for subsequent functional and evolutionary analyses. A more detailed discussion of the species-distinctive loci and their possible ecological role is provided in the Supplemental Information, along with a screen for large-scale genomic divergence between males and females in *L. planeri*.

In summary, we show that the sympatric lampreys *L. fluviatilis* and *L. planeri* are genetically highly distinct, and that the regions of strongest divergence contain several candidate genes for adaptation to a migratory versus resident life-style.

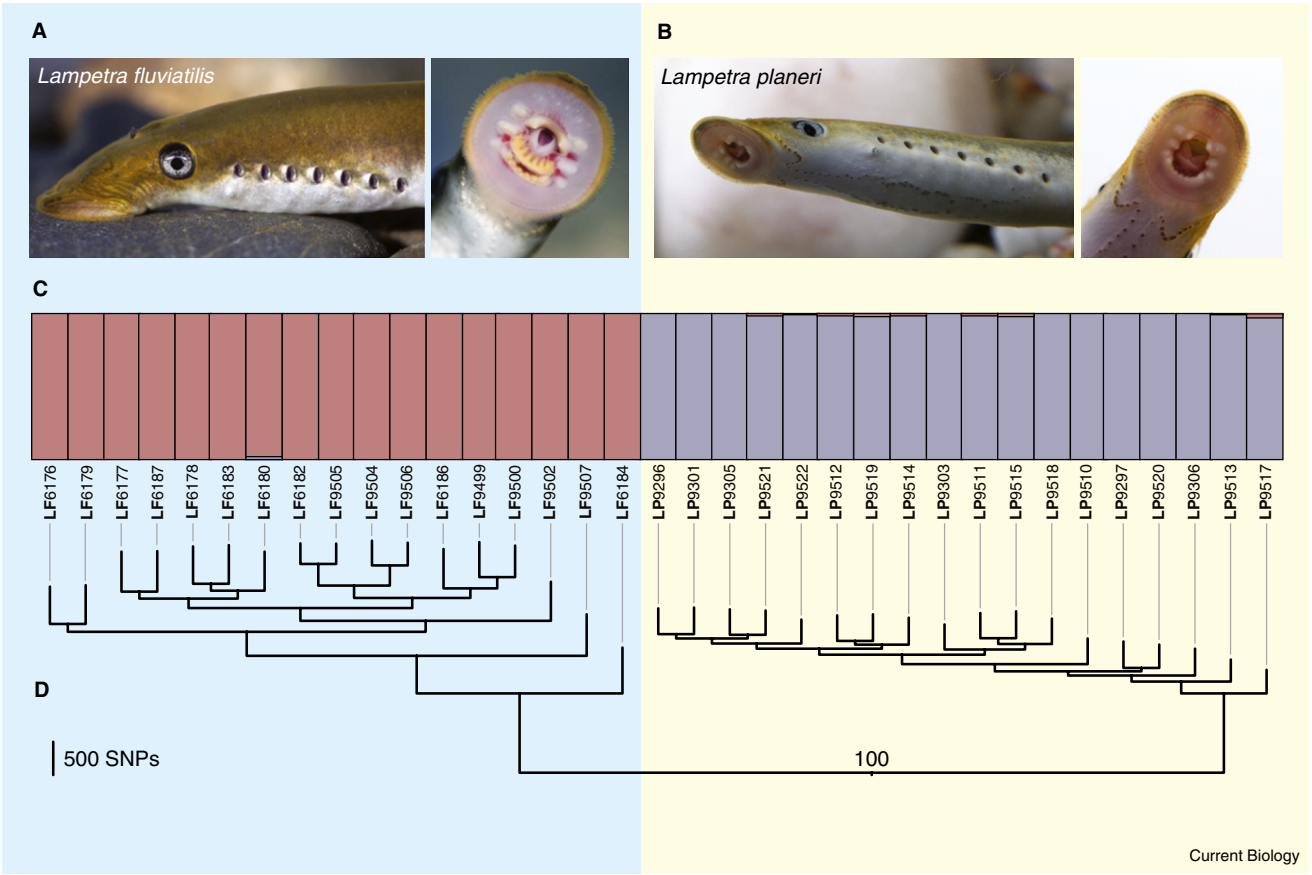

Figure 1. Genetic divergence in a lamprey species pair.
The European river lamprey (*Lampetra fluviatilis*) (A) and the brook lamprey (*Lampetra planeri*) (B) are morphologically distinct in the adult stage. (C) A Bayesian population assignment test with Structure and a subsequent evaluation with Structure Harvester reveal the existence of two clusters ($K = 2$) in our SNP dataset, corresponding to the two sympatric species *L. fluviatilis* and *L. planeri*. Each bar represents the assignment probability (0 to 1) of a single specimen to one of these two clusters (color coded in red and purple, respectively). (D) Phylogeny of the 35 lamprey specimens from the Sorraia River in Portugal based on 14,691 SNPs and maximum parsimony in PAUP* (heuristic search with stepwise addition, TBR branch swapping and allowing polymorphisms). The specimens are grouped into two clades, which exactly match the two species *L. fluviatilis* and *L. planeri* (the bootstrap value for the basal branch is provided).

Supplemental Information

Supplemental Information including experimental procedures, a figure and a table can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.06.026.

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